Ⅰ.Oligo

1. PCR System

Name	Total volume: 25μL	Total volume: 50μL
10*rTaq Buffer	2.5μL	5μL
2.5MdNTP	2μL	4μL
Oligo mix(（1μM，Each primer 1/36μM）	9μL	18μL
rTaq	0.13μL	0.25μL
ddH2O	Add to total volume.	Add to total volume.

1. Procedure

94℃，5min
94℃，30s
55℃，30s ×30
72℃，1min/kb
72℃，7min
4-12℃，∞

(The 94℃ 30s, 55℃ 30s and 72℃ 1min/kb steps are bracketed together ×30.)

3. Gel Analysis

Ⅱ.Overlap PCR Protocol 1

1. Amplify both two DNA fragments by conventional PCR.
2. Mix 50-100ng fragment Ⅰ with equimolar fragment Ⅱ in an Eppendorf tube.
3. Common PCR using the mixture in step 2 as templates.
4. Gel analysis.

Ⅱ. Overlap PCR Protocol 2

1. Amplify both two DNA fragments by conventional PCR.
2. Mix 50-100ng fragment Ⅰ with equimolar fragment Ⅱ in an Eppendorf tube.
3. Conventional PCR without primers using the mixture in step 2 as templates. Run for 8-12 cycles.
4. Conventional PCR using the product in step 3.Run for 25-35 cycles.
5. Gel analysis.